



Discovery of Biomolecular Indicators for Force Health Protection

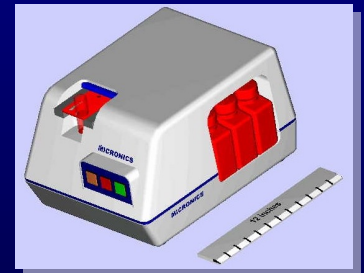
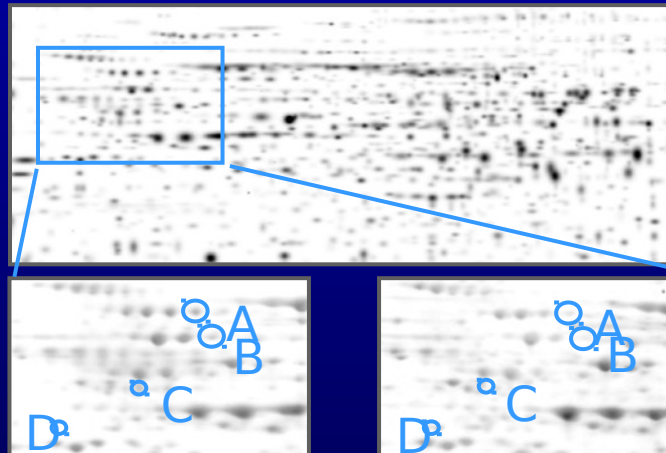
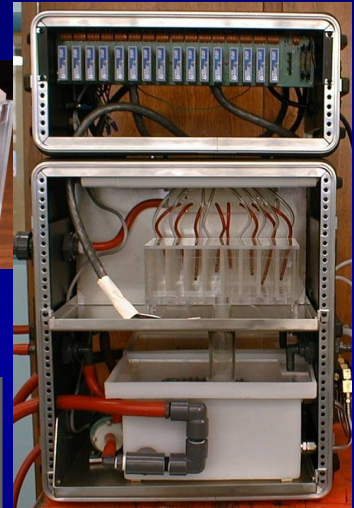
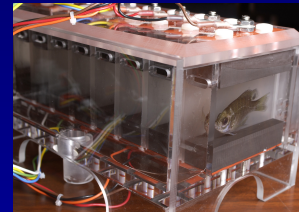
**LTC Rodger K. Martin, Ph.D.
US Army Center for Environmental Health Research
Fort Detrick, Maryland**

January 28, 2004



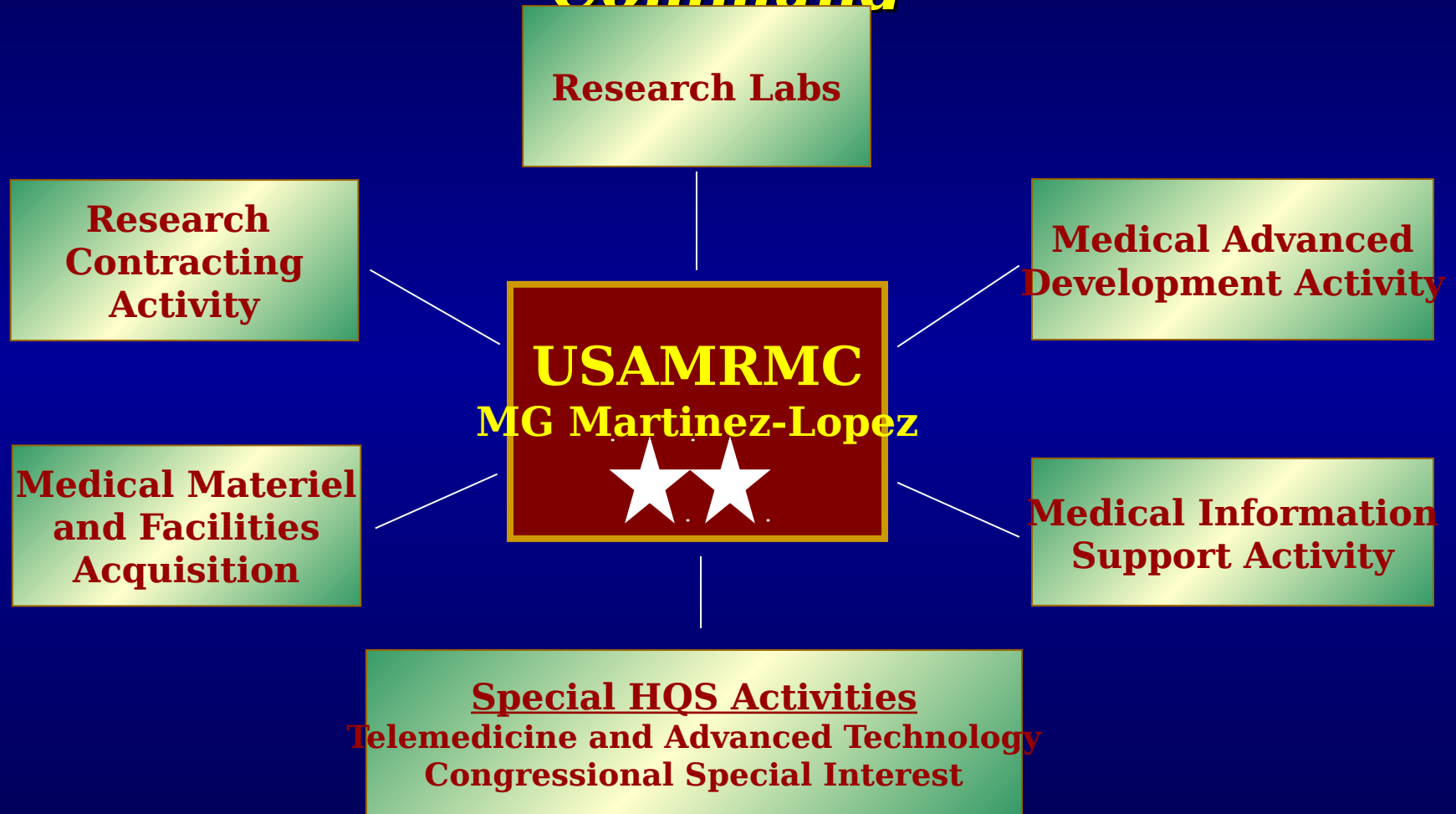
Outline

- ❖ Introduction
- ❖ *USACEHR* Mission
- ❖ Organization
- ❖ Drivers
- ❖ Research
- ❖ Conclusion





U.S. Army Medical Research & Materiel Command

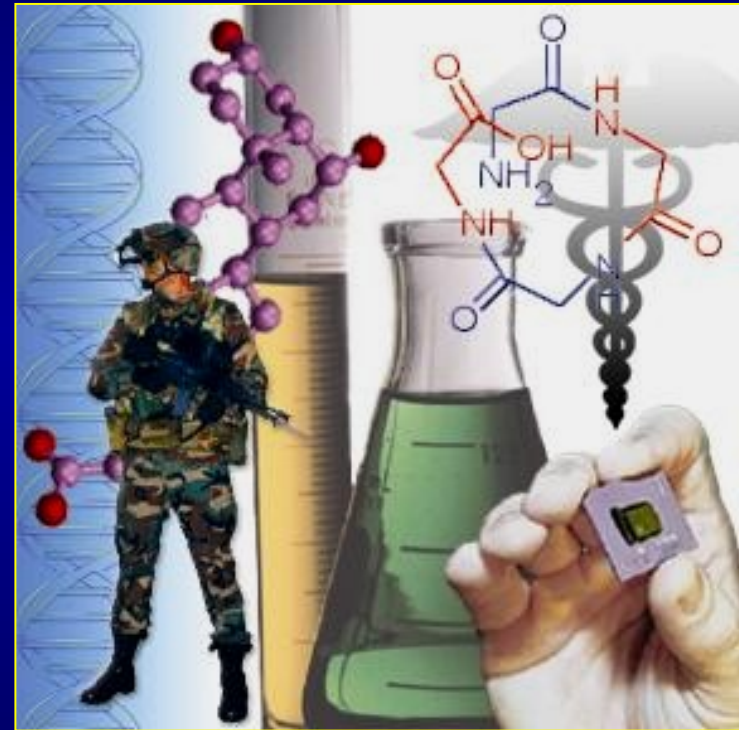


USACEHR is a Subordinate Command of USAMRICD



USACEHR Missions

- ❖ **Enhance Force Health Protection and Deployment Health Surveillance**
- ❖ **Protect the soldier from environmental hazards - OEHS**
- ❖ **Provide early warning of environmental hazards**
- ❖ **Develop new water and food testing technologies.**





Occupational & Environmental Health Threats

Deployment Health Surveillance

- Treat Casualties
- Diagnose Conditions
- Investigate Case Clusters

- 1 Identify Hazard
- 2 Conduct Health Risk Assessment
- 3 Implement Controls
- 4 Monitor Compliance





OEHS Research Mission Drivers

Gulf War

Somalia

HaitiBosnia

Kosovo

OEF

OIF

AR 40-5

1990

1991

1992

1993

1994

1995

1996

1997

1998

1999

2000

2001

2002

2003

2004

AR 40-10

ASD (HA) Memo

TRADOC Pamphlet 525

DA Memo

ORD for FCS

NSTC PRD-5

DOD Directives 6490

JCS Memo

ASD (HA) Memo

**DOD Directives 6490
(revised)**



Top Ranked OEHS R&D Needs



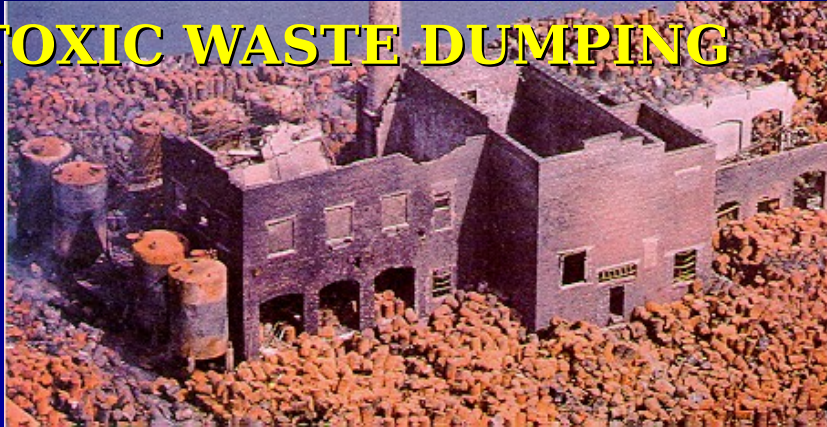
- ❖ **Improved Sampling and Analysis Equipment**
- ❖ **Development of Biomarkers of Exposure, Effect, and Susceptibility**
- ❖ **Enhanced Individual Exposure Monitoring and Documentation**
- ❖ **New Chemical Hazard Assessment Models**

ASD (HA) Memorandum, Subject: Enhanced Science and Technology Support for Occupational and Environmental Health Surveillance (OEHS), June 2003



Toxic Chemical Threats

TOXIC WASTE DUMPING



OPERATIONAL ACTIVITY

ENVIRONMENTAL WARFARE

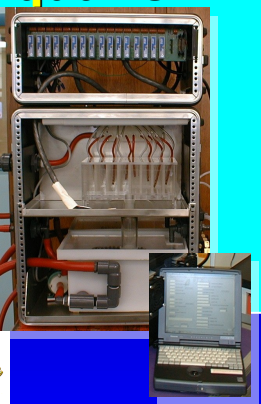


INDUSTRIAL ACTIVITY

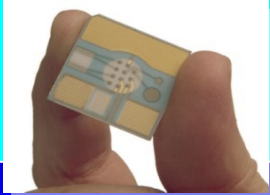
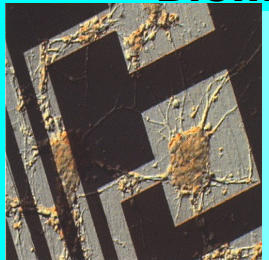


BIOMONITOR DEVELOPMENT

Aquatic Biomonitoring



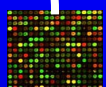
Environmental Sentinel Biomonitor



BIOMARKER DEVELOPMENT

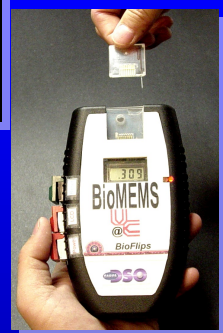
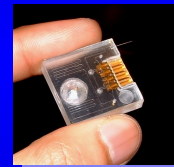


**Biomarkers
Exposure
Effect
Susceptibility**



Biomarker Applications (6.2/6.3)

- Input to ESB
- Soldier diagnostics
- Phase I Studies

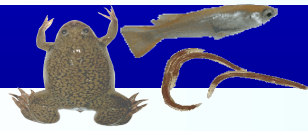


Biomarker Pre-Clinical Validation (6.2)



- Mammalian animal models
- Validation Studies

Biomarker Discovery (6.1)



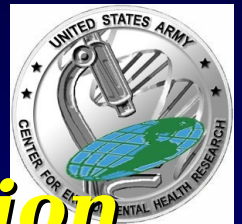
Alternative species use: Tools:

- High throughput
- Hypothesis generation

- Genomics/proteomics
- Bioinformatics

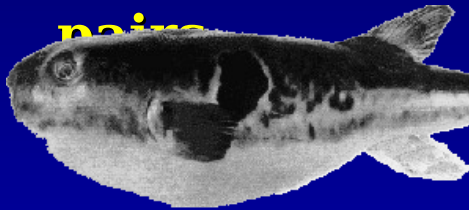
Leveraging:

- CB
- Others...



Paradigm Shift...Genomics Revolution

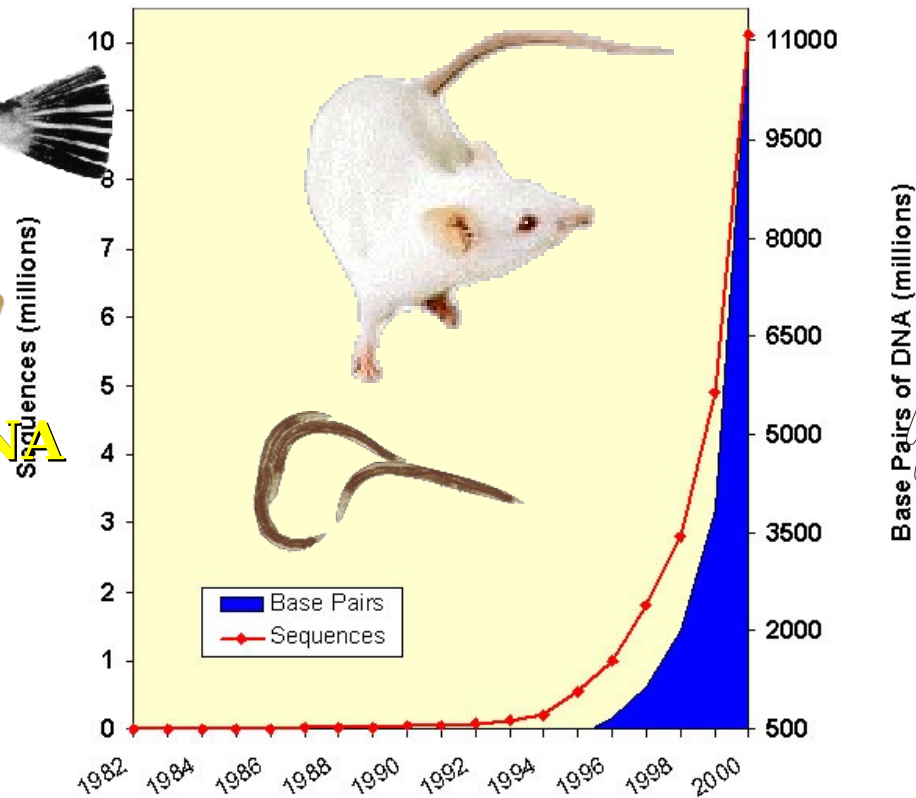
**~400 Million
DNA base
pairs**



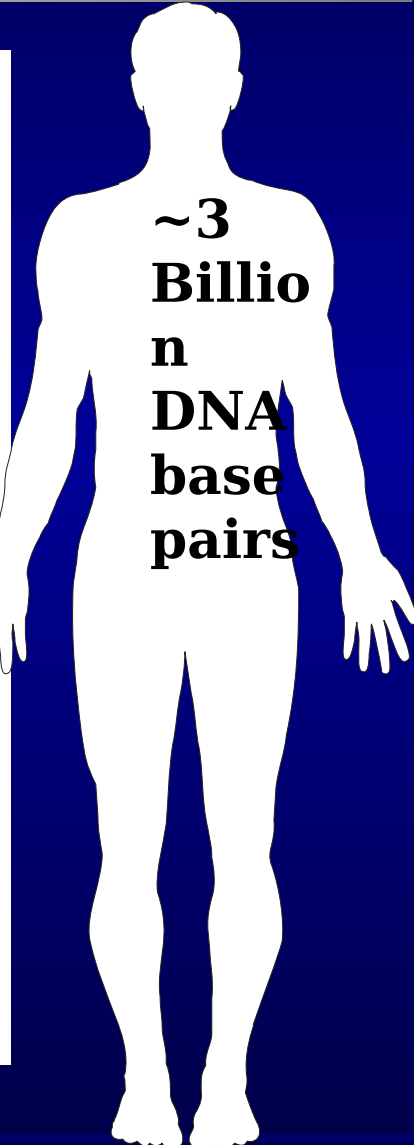
**~1.7 Billion DNA
base pairs**



Growth of GenBank

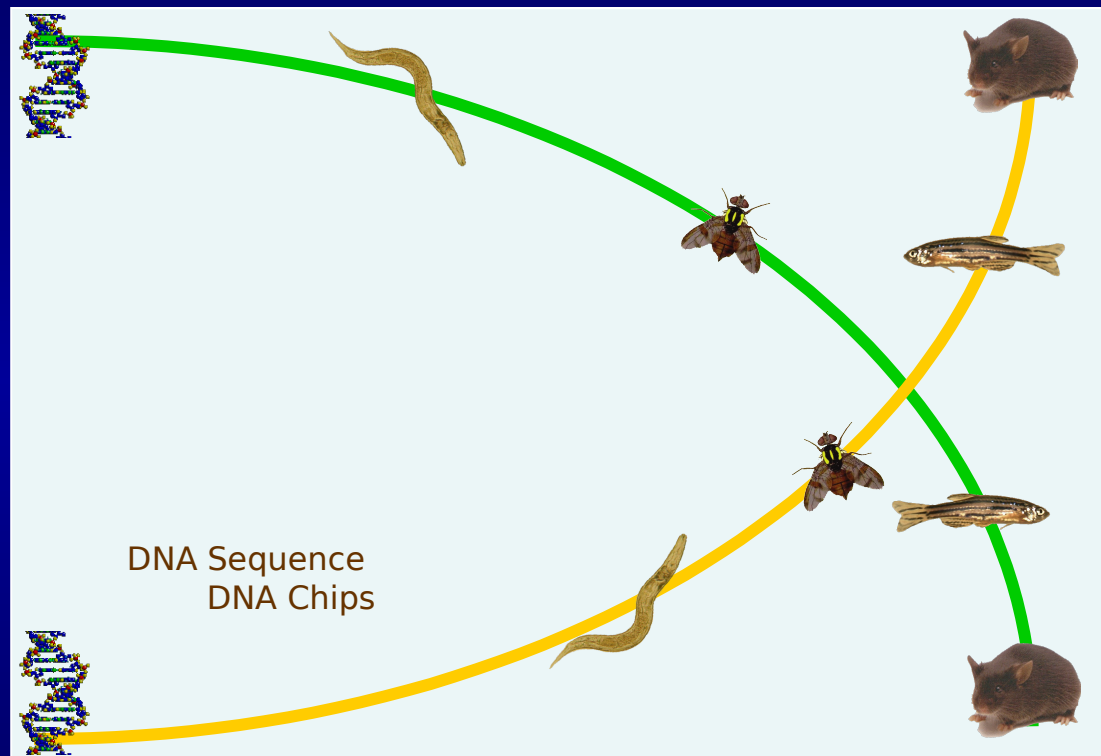


**~3
Billion
DNA
base
pairs**





Use of Alternative Species



**Applicability
to
Humans**
**Affordability
of Gene
Analysis**

Usefulness for Toxicant -Target Validation

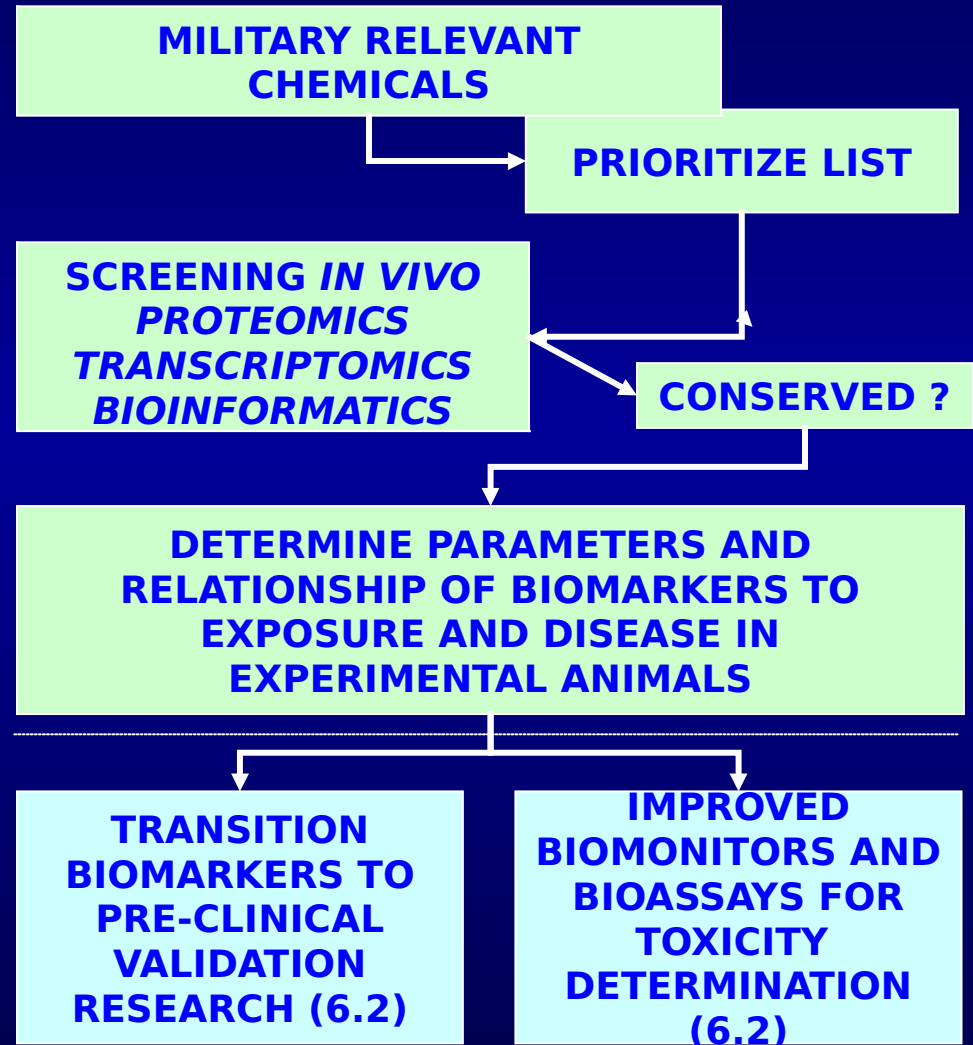
(Adapted from Hackett and Essner,
Genetic Engineering News, 2003)



Discovery of Biomolecular Indicators for Force Health Protection

Strategy & Rationale:

- Establish a prioritized list of MRCs/TICs/TIMs from myriad of threats
- Rapidly screen substances in model species/cell culture at
 - RNA expression levels
 - Protein expression levels
- Select candidate protein biomarkers based on function and conservation
- Establish parameters and relevance of biomarkers to mammalian exposure and disease
- Transition biomarkers to preclinical validation biomonitor



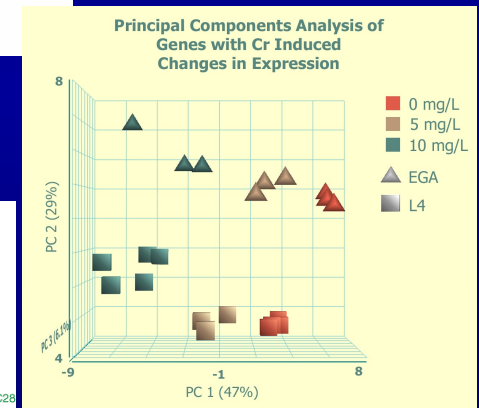
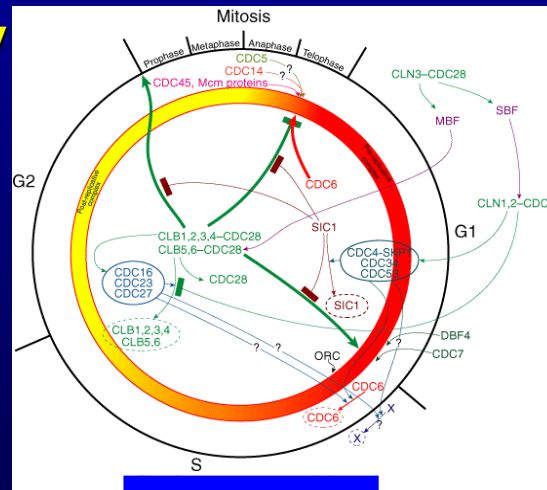
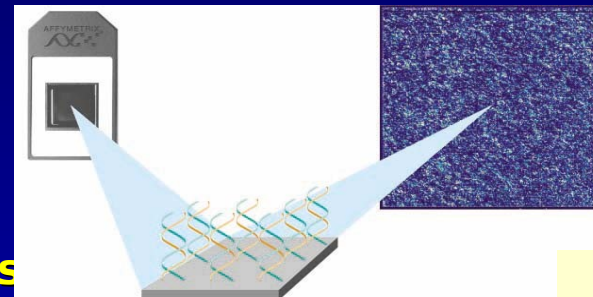


Discovery of Biomolecular Indicators for Force Health Protection

Expression Data to Physiology

Methods/Techniques:

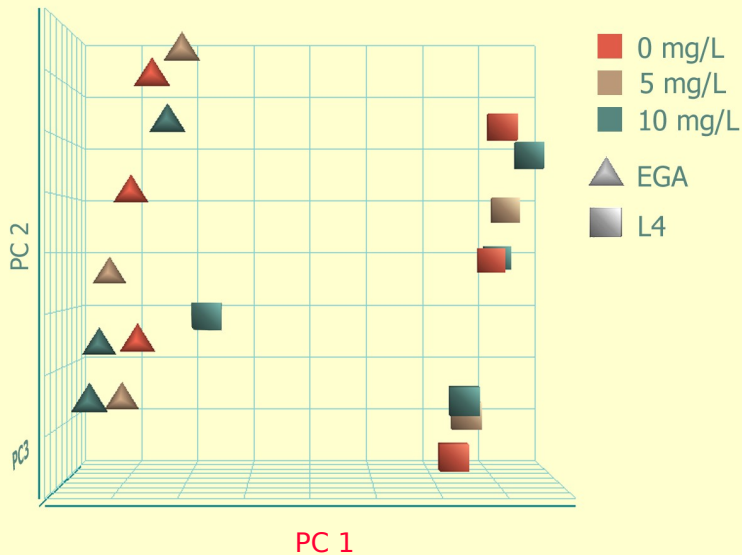
- Use of model species/
cell culture
- Transcriptomics
 - Affymetrix GeneChips
- Proteomics
 - 2D Gel Electrophoresis
 - 2D HPLC/Mass Spectrometry
- SELDI-TOF
- Bioinformatics
- Cell modeling
- Pathway prediction





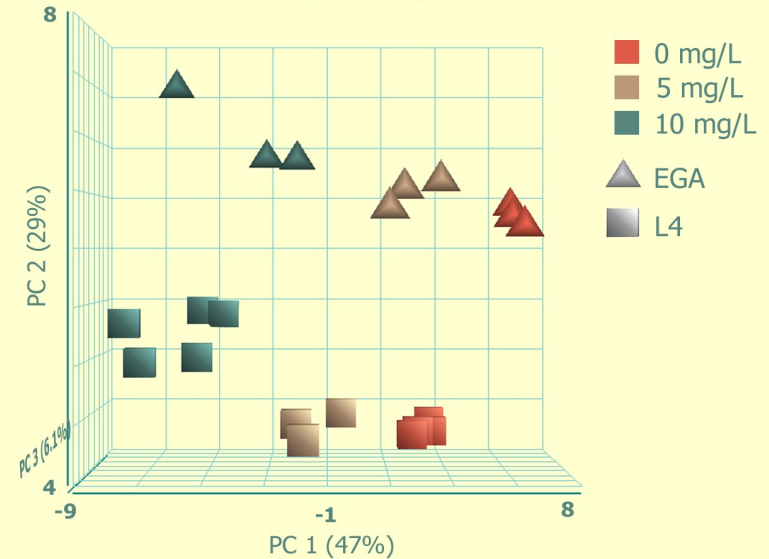
Discovery of Biomolecular Indicators for Force Health Protection

Principal Components Analysis
of Expression of All Genes



Whole Chip
22,500
genes

Principal Components Analysis of
Genes with Cr Induced
Changes in Expression



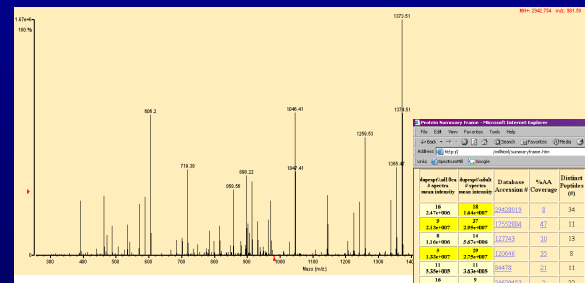
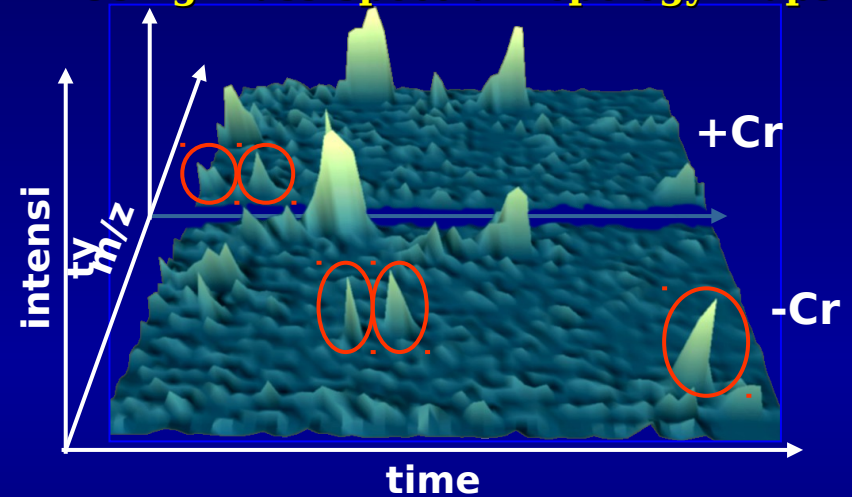
ANOVA ($p < 0.001$)
45 genes



Discovery of Biomolecular Indicators for Force Health Protection

- Amount of data to be managed and analyzed is greater for the proteome vice the transcriptome
- The proteome is more complex
- The relationship between protein and transcript expression levels is not rigidly predictable.
- Tools for accurate and efficient identification of proteins from MS data are needed (especially for organisms whose sequences are not fully characterized)
- Lack of quantitative

Comparison of Protein Expression Using Mass Spectral Topology Maps



Agilent's Spectrum Mill MS Sequence Search Tool

The screenshot displays the Agilent Spectrum Mill software interface. On the left, a list of search results is shown with columns for 'Protein Name', 'Accession', 'Score', and 'Rank'. On the right, a 'Filter' panel allows users to refine results based on various criteria such as 'Protein Name', 'Accession', 'Score', and 'Rank'. The interface includes various input fields, checkboxes, and buttons for navigating through the search results.

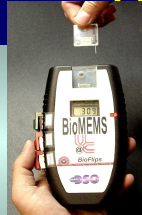


Management and Integration Strategy

Technical Performers

DARPA
Industry

Biochip
Platforms



Incorporate
Classic
Biomarkers

Incorporate New and
Improved Biomarkers

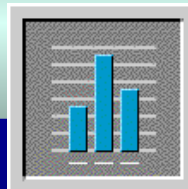
Products

Biomarker &
Biomonitoring
Systems

NIOSH
EPA
NIEHS
CDC
NHRC
USACHPPM
USACEHR

Human
Epidemiologic
al
Studies

Classic
Biomarkers



New
Biomarkers

Human
Biomarkers Validation

Validated
Biomarkers

WPAFB
NIOSH
EPA
NIEHS
Academia
USACEHR

Biomarker
Discovery
Research

Using
Mammalian
Animals

Pharmacokinetics
time-course



Homologous
Biomarkers

Mammalia
n Animal
Validation

Phenotypi
c
Anchoring

Improved
&
Predictive
Testing
Methods

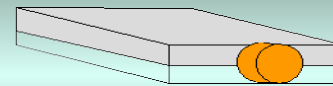
USACEHR
NIH
Academia

Biomarker
Discovery
Research

Using *in vitro* and
non-
mammalian
animals



Homologous
Biomarkers



Phenotypi
c
Anchoring

Validated
Alternative
Testing
Methods



Conclusion

USACEHR...

***...a history of providing material
solutions for FHP and OEHS
requirements***

Solutions for Today - Research for Tomorrow



Gulf War Illnesses Research

The Force Health Protection program is based on the medical lessons learned from the Gulf War and later deployments. The federal government has funded more than 200 research projects focused on Gulf War Illnesses, at a cost of approximately \$224 million. This important deployment health research must continue.”

Dr. William Winkenwerder Jr., Assistant Secretary of Defense for Health Affairs (USAToday, 14 July 2003)



Back-up Slides



USACEHR Relevance

- ~~OEHS Surveillance requirements are increasing~~
- **RDT&E capabilities for OEHS Surveillance are not available at ICD or elsewhere in DoD**
- **Need subject matter expertise to help manage grants, coordinate research efforts, and to represent MRMC at IOM, JESWG, Tri-Service Toxicology Committee, etc.**

Provide new:
• **Leverage new biotechnology advances for development of new screening methods and risk assessment methods and devices,**

dose response models, exposure assessment methods (dose estimation), and risk characterization

**CEHR
Products**

Operational Risk Management

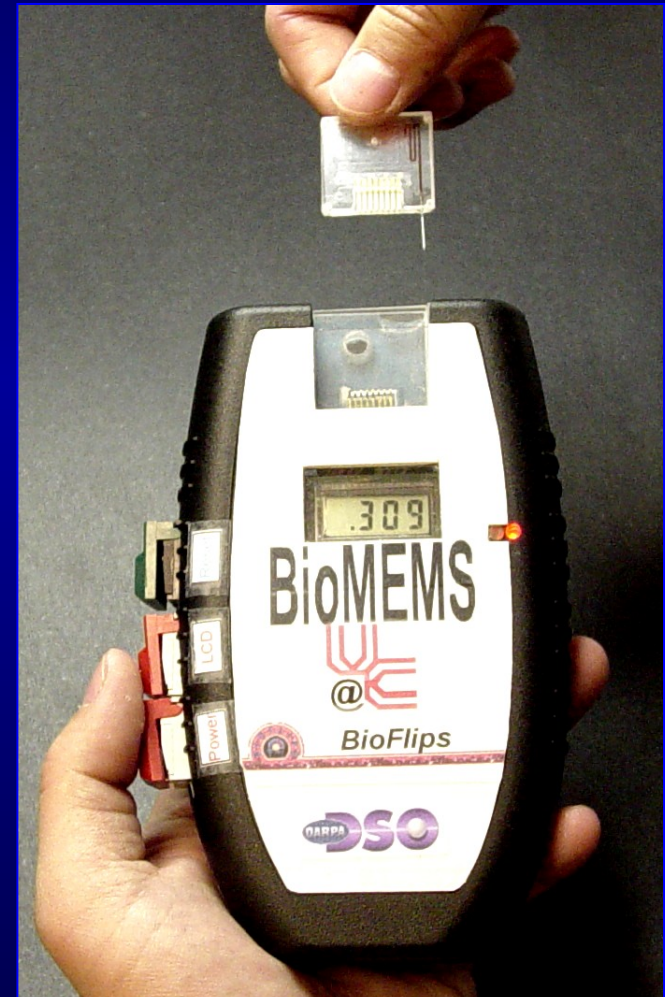
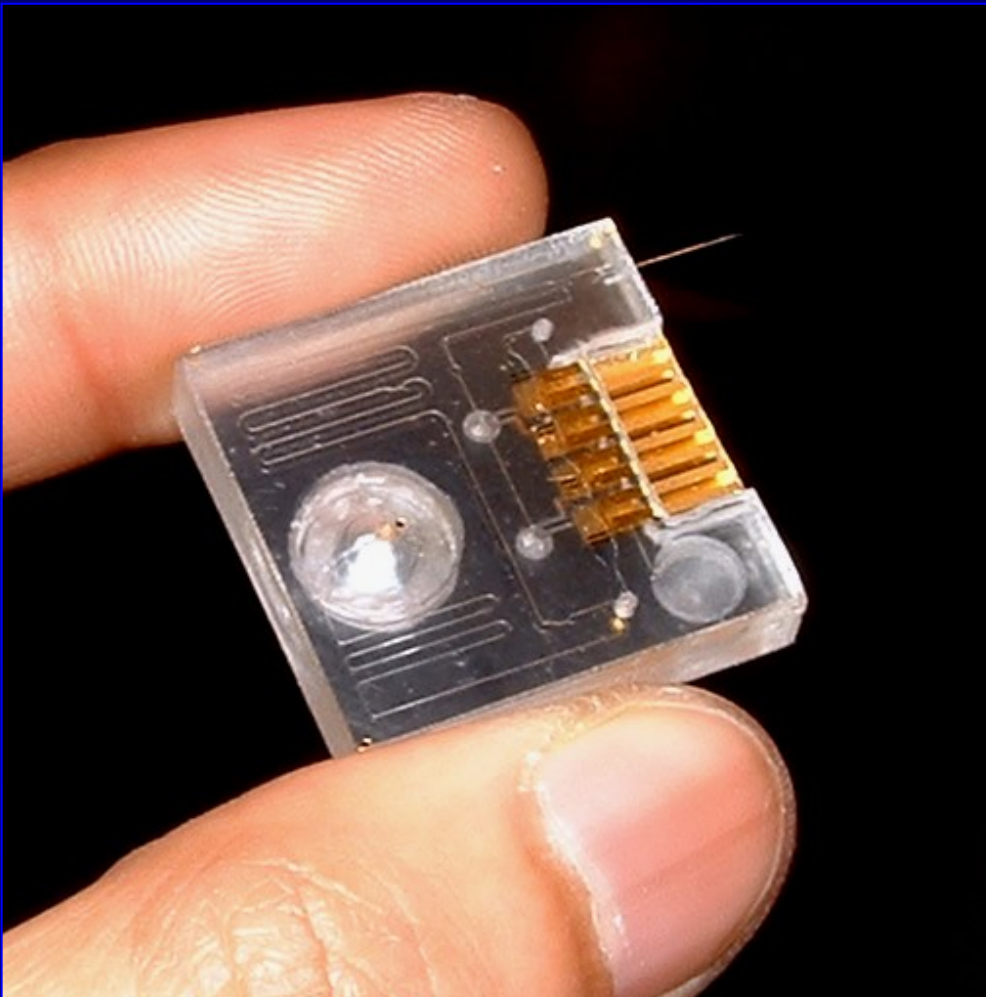
- 1** Identify Hazard
- 2** Conduct Health Risk Assessment
- 3** Implement Controls
- 4** Monitor Compliance

Especially for chemical mixtures and

USACEHR: Relevant and Ready

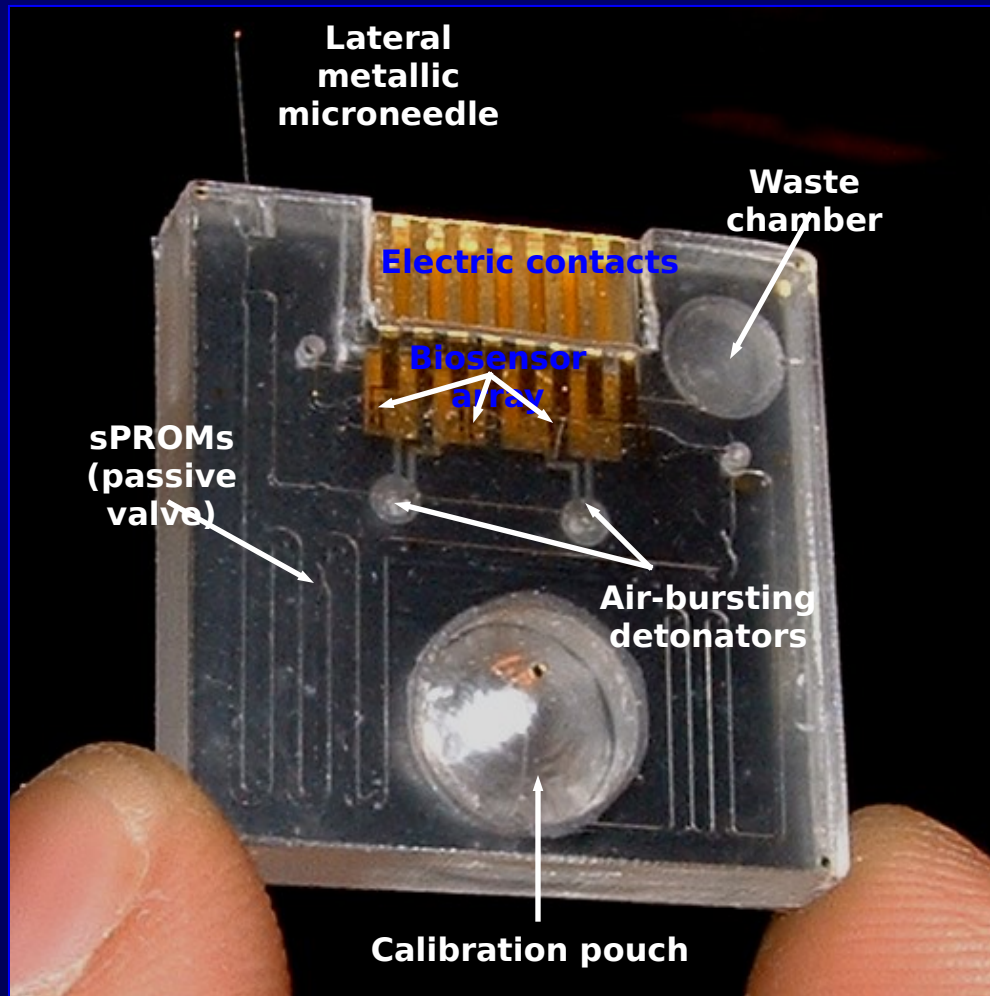


Plastic-Based Structurally Programmable Microfluidic Biochips





Disposable Smart Biochip Cartridge



- Fully integrated disposable smart biochip cartridge
- Lateral metallic microneedle for biofluid sampling
- On-chip calibration function
- Multi-analyte detection (PO_2 , glucose, and lactate)
- Multiple air-bursting detonators as on-chip pressure source
- Dimension: 1" x 1" x 0.25"



Partnerships and Leveraging

- **Environmental Protection Agency (2x Interagency Agreements, IAGs)**
- **National Institute for Occupational Safety and Health (IAG)**
- **National Institute of Environmental Health Sciences (MOU)**
 - » National Center for Toxicogenomics
 - » Chemical Effects in Biological Systems (CEBS) Knowledge Base
 - » Proteomics Contract with Large Scale Biology Corporation
 - » Interagency Coordinating Committee for the Validation of Alternative Methods
- Food and Drug Administration
- CRADAs with GEO-CENTERS, Inc., and New York City
- **U.S. Army Corps of Engineers (Reimbursable agreement)**
- ***U.S. Army Center for Health Promotion and Preventive Medicine***
- **U.S. Army Medical Research Institute of Chemical Defense**
- **U.S. Army Medical Research Institute of Infectious Diseases**
- **Walter Reed Army Institute of Research**
- **Tri-Service Toxicology Consortium, Wright-Patterson, AFB**
- Frederick NCI/MRMC/NIAID Combined Bioinformatics and Chemoinformatics Forum
- Mount Desert Island Biological Laboratory (Aquatic Toxicogenomics DB)
- International Life Sciences Institute, Genomics/Proteomics Committees
- Virginia Bioinformatics Institute (DOD contract)
- **U.S. Army Soldier Biological and Chemical Command**
- **Army High Performance Computing Research Center**